Docket No.: 28079/41333
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Kenneth A. Barton et al.

Application No.: 07/827,906 | Confirmation No.: 3375

Filed: January 30, 1992 | Art Unit: 1638

For: IMPROVED EXPRESSION OF GENES IN PLANTS | Examiner: A. R. Kubelik

DECLARATION UNDER 37 C.F.R. § 1.132

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, JAMES A. BAUM, Ph.D., declare as follows:

I. BACKGROUND, QUALIFICATIONS, AND DISCLOSURE OF INTEREST

1.1 I received my Ph.D. in Genetics from North Carolina State University in 1981. For the following six years, I served as a postdoctoral research fellow at the University of Georgia. I joined Ecogen as a research scientist in 1987. I held several positions within Ecogen, including Principal Research Scientist and Director of *Bacillus thuringiensis* (*B.t.*) research in 1995-1999 and Supervisor of the Ecogen-Monsanto Research & Development Program in 1996-1999. I have been employed as a Research Scientist at Monsanto since 1999. My research has included the discovery of novel insecticidal protein genes from *B.t.*, the engineering of improved insecticidal proteins, the study of DNA-protein interactions, the regulation of gene expression in *Bacillus*, and the optimization of *cry* gene expression in *B.t.* Throughout my career, I have worked alongside and supervised research scientists and postdoctoral fellows engaged in insecticide research. My academic and

professional experience, as well as my research interests, is more fully detailed in my *Curriculum Vitae*, which is attached as Exhibit A.

1.2. Monsanto asked me to comment on the disclosure of Barton et al., U.S. Patent Application No. 07/827,906 ("the Barton application") from the perspective of a research scientist practicing in the field at the time that the Barton application was filed. I was instructed that the Barton application was first filed in August 1989. Therefore, my remarks focus on the perspective that an average research scientist would have had in August 1989.

1.3. Monsanto is my current employer and I understand that Monsanto owns the Barton application. Monsanto provided me with a copy of the Barton application; a copy of the Office communication dated February 25, 2009 ("the Action"), in which the U.S. Patent and Trademark Office's patent examiner set forth her current reasons for rejecting the current claims in the Barton application; and a copy of the set of claims of the Barton application that were pending on February 25, 2009, and considered by the patent examiner. (Exhibit B.) I have reviewed these materials for the purposes of this declaration.

II. WRITTEN DESCRIPTION OF THE BARTON APPLICATION

2.1. I understand that the patent examiner has rejected claims under the "written description" section of the patent laws and taken the position that a person of average skill in the field of the invention would not have recognized that the inventors were in possession of certain aspects of the invention, as presently claimed, at the time the application was filed. In particular, the Action states, "The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." (Action at page 3.)

A. The written description requirement

2.2. It has been explained to me that the goal of the written description requirement is that the patent conveys that an applicant has invented the subject matter which the applicant claims as the invention. I understand that another objective of this requirement is to put the public in possession of the invention that is claimed. It has been explained to me that an applicant shows possession of the claimed invention by describing in the patent application the claimed invention using descriptive means such as words, structures, figures,

diagrams, tables and formulas that fully set forth the claimed invention. I understand that there is no requirement that the language of the claims be found *word-for-word* in the application, as long as the subject matter that is claimed is supported in the application as a whole, through some combination of express, implicit or inherent disclosure.

2.3. I understand that whether the written description requirement is met is viewed from the standpoint of a person of average skill in the field of the invention at the time that the application was filed, with the understanding that the application is written for such persons and the specificity of the disclosure need not be as detailed with respect to features within the knowledge and skill of such persons. Thus, I understand that information which is well known to those in the field of the invention need not be described in detail in the application.

B. The Barton application conveys that modifying codons for "at least the first 25 amino acids" and "at least 59 amino acids" is part of the invention.

3.1. I understand that one of the patent examiner's rejections concerns whether the Barton application describes the variation of the invention found in the claims wherein a starting coding sequence is modified by replacing codons for at least the first 25 amino acids or at least 59 amino acids in the 5' end of a starting coding sequence with codons for identical amino acids that have the highest frequency of use in plant genes. The patent examiner's analysis is reflected by the following excerpt from the Action at page 8:

> Neither the instant specification nor the originally filed claims appear to provide support for modifying the codons for at least the first 25 amino acids in any coding sequence as in claims 28, 33 and 40. The specification only provides support for modifying "about 25 codons" at the N' terminal end of the Bt sequence used in the Examples (pg 13, lines 15-22).
>
> Neither the instant specification nor the originally filed claims appear to provide support for substituting at least 59 amino acids in any coding sequence as in claim 29. The specification only provides support for modifying 59-138 codons of the Bt sequence used in the Examples (pg 13, line 9).

3.2 In my opinion, it would have been clear to a reader of the Barton application in 1989 that the inventors intended the invention to encompass more variations than those quoted by the examiner in the Office Action ("about 25 codons" or "about 59-138 codons" of the 5' end of a starting coding sequence). For example, at page 13, lines 8-26, the Barton application describes the invention as follows:

> [T]he coding region of the protein expression cassette was altered by **as few as 59** to as many as 138 codons, all at the amino terminal end of the protein or the 5' end of the coding region. Since the results did not seem to vary greatly based on the length of the substituted codons, it is possible that the **increased expressional efficiency is due principally to the substitution at the amino-terminal, or 5', end of the coding sequence**, perhaps those in the first 25 amino acids. . . . [T]his would suggest that entire coding regions need not be altered to gain a relatively significant increase in efficiency of expression, merely the amino-terminal end of the coding region, for perhaps about 25 codons. **Performing such a codon substitution for the remaining portion of the coding region might still be expected to increase efficiency of expression**, although perhaps less dramatically.
>
> (Emphasis added in bold.)

3.3 This excerpt from the Barton application clearly conveys that, according to one statement of the invention, expression enhancement is achieved with substitutions at the 5' end of the coding sequence with plant preferred codons. It also clearly conveys variations of the invention where "the first 25" or "about" the first 25 are substituted.

3.4. The quoted passage also conveys that the inventors contemplated *additional* codon substitutions (beyond the first 25) codons to *increase efficiency of expression*. It is abundantly clear when one sentence refers to altering "about 25 codons" and the very next sentence refers to performing codon substitutions for the remaining portion of the coding region to improve results that the invention includes modifying "at least the first 25" codons. Performing (about) 25 substitutions, or performing (about) 25 plus additional substitutions, conveys to the reader that substituting *at least* the first 25 is one definition of the invention. The application also has examples (alteration of as few as 59 to as many as 138) that make it clear that "at least 25" is another way to define the invention. In the Examples, three plasmid constructs were generated which substituted the first 59, 104, and 138 codons of a *Bacillus thuringiensis* (*B.t.*) delta endotoxin gene with codons selected from

Figure 1 of the application with the highest frequency of use. (See Barton application at page 10, lines 3-11; and page 13, line 30, through page 17, line 25.) The plasmids were introduced into tobacco, and resistance against tobacco hornworm was observed. In plants exhibiting insecticidal activity, the plants comprising the synthetic sequences exhibited more uniform and greater toxicity compared to non-modified sequences, which indicates that the modified sequences were expressed more efficiently than the non-modified sequences. (See Barton application at page 19, line 7, through page 21, line 9.)

3.5. It is also clear from the Barton application that substituting codons for at least 59 amino acids in the 5' end of the coding sequence was one definition of the invention. The inventors explain in the quoted excerpt that they had data that as few as 59 to as many as 138 at the 5' end produce desirable results. Thus, the reader would have understood that making 59-138 substitutions at the 5' end was another way of defining the invention. The quoted excerpt also refers to performing codon substitution for the remaining portion of the coding region, which would be understood to be a statement that codons beyond position 25 or 59 or 138 could be substituted. Thus, the reader would have understood that making *59 or more* substitutions (which is another way of saying "at least 59") is another preferred variation of the invention. Since the inventors contemplate making as few as 59 to as many as 138, and also contemplate performing substitutions in the remaining portion of the coding region, it is clear that 138 was not intended to be an upper limit on the number of changes.

3.6. To summarize, the Barton application, including the excerpt quoted above, would have conveyed to a reader in 1989 that the invention had several variations, including the variations defined by the rejected claims, including claims 28, 29, 33, and 40, for example.

C. The Barton application provides basis for modifying any coding sequence.

3.7. I understand that the Patent Office also rejected claims because the patent examiner raises doubts about whether the Barton application describes the practice of the invention wherein the 5' end (e.g., at least the first 25 codons or 59 codons) of a coding sequence other than the *B.t.* delta endotoxin coding sequence is modified. The patent examiner proposes that the "particular numbers" of codon substitutions "are mentioned only in the context of the examples, not in a context that says that substitutions of the first 25, 59,

or 138 codons could be applied to any coding sequence." (Action at page 4.)

3.8. In my opinion, it would have been clear to a scientist in the field that the teachings in the Barton application to modify the codons for at least the first 25 amino acids or at least 59 amino acids in the 5' end of the coding sequence applies to *any* starting coding sequence, not just the coding sequence used in the Examples. My analysis is based on several passages in the Barton application which highlight the broad applicability of the invention. For example, the Barton application explicitly teaches that the invention, exemplified in the context of a *B.t.* delta endotoxin gene, is equally applicable to other prokaryotic or eukaryotic coding sequences:

> It also becomes obvious to one skilled in the art that the method is used with the particular procaryotic gene described and illustrated in the present invention **is equally applicable to other procaryotic or even eukaryotic, genes which happen not to express well in plants.** The results of this procedure demonstrate that at least one factor in the relatively low expression level of the prokaryotic B.t. protein in plants is due to the actual makeup of the codon usage pattern of the particular prokaryotic gene. **Other procaryotic or eukaryotic genes which similarly use a large number of codons which are not among those preferentially expressed by plants may also be altered in the similar fashion**. . . Therefore it is possible to **express many foreign proteins effectively and efficiently in plant cells** and still to produce a protein identical in amino acid sequence to the native protein while still gaining the efficiencies possible using the transcriptional and translation machinery of plants more effectively.
>
> (See the Barton application at page 12, lines 8-29 (emphasis added).)

3.9. A typical scientist in the field would have understood the above passage to mean that all features of the Barton application's method, regardless of where they are described in the application, are applicable to foreign genes other than *B.t.* delta endotoxin genes, particularly genes in which enhanced expression in plants is desired. This understanding is supported by the application at page 2, lines 9-23, which refers to the difficulty in expressing *B.t.* delta endotoxin as "an example" of reduced heterologous gene expression in plants, and page 21, lines 15-26, which states that the invention is not limited to the single illustrative practice of the invention provided in the Examples. Indeed, the application characterizes the invention as a method for constructing chimeric coding sequences for expression in plant cells by modifying the native coding sequence for *a foreign*

gene. (Specification at page 3, lines 24-33.) It is clear that "foreign gene" is used in the Barton application in a generic sense to refer to "other prokaryotic or eukaryotic genes," and is not limited to *B.t.* delta endotoxin genes.

3.10. At page 4 of the Action, the patent examiner pointed to a particular passage in the Barton application at page 13, lines 7-16, which discusses modification of codons for at least the first 25 amino acids or at least 59 amino acids. The passage is found in the Barton application just prior to the Examples. The particular location of the discussion does not convey that the disclosure is limited to the particular sequence used in the Examples. On the contrary, a reader would understand the discussion at page 13, lines 9-26, to be an analysis of the Examples that is applicable to other prokaryotic or eukaryotic coding sequences. For example, the application states, "[a]s an examination of the following Examples will reveal to one skilled in the art, the substitution of plant preferred codons in **a plant expression cassette** results in an increased level of efficiency in expression of the engineered protein." (Specification at page 13, lines 3-7 (emphasis added).) The application further states, "this would suggest that **entire coding regions** need not be altered to gain a relatively significant increase in efficiency of expression, merely the amino-terminal end of the coding region." (Specification at page 13, lines 18-22 (emphasis added).) It is clear to the reader that the teaching to modify the 5' end of a coding sequence is a general teaching – not limited to a specific sequence in the Examples, but applicable to any coding sequence, as specified in the claims.

3.11 Original claim 10 of the Barton application confirms that the inventors intended the disclosure of 5' modifications to apply to any coding sequence. Original claim 10 is directed to a transgenic plant comprising a chimeric gene coding for the expression of a foreign protein. The chimeric gene's coding sequence differs from that of the foreign gene in a segment *at the 5' end of the coding region.* (See Barton application at page 24, original claim 10.) The claim language is not limited to particular chimeric genes or foreign proteins; instead, it is clear that inventors' method included modifying the 5' end of any coding sequence.

3.12 Thus, in my opinion, it is plain from the Barton application that the inventors specifically contemplated modifying codons for at least the first 25 amino acids or at least 59 amino acids at the 5' end of *any* coding sequence, and *B.t.* delta-endotoxin is

described in the Examples as only an example of a foreign gene that is expressed poorly in plant cells.

III. CERTIFICATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

Date 8-12-2009

James A. Baum, Ph.D.

EXHIBIT A

James Arthur Baum

Research Fellow

Monsanto
700 Chesterfield Parkway West
Chesterfield, MO 63017

Professional History

6/15/87 - 9/1/88	Research Scientist, Ecogen, Inc.
9/1/88 - 9/1/89	Senior Research Scientist, Ecogen Inc.
11/1/89 - 11/1/91	Project Leader: Ecogen/DOW-ELANCO research program
9/1/89 - 3/15/95	Team Leader: Strain Development, Ecogen Inc.
2/1/91 - 2/16/99	Principal Research Scientist, Ecogen Inc.
3/15/95 - 2/16/99	Director of *Bt* Research Principal Research Scientist, Ecogen Inc.
1/24/96 - 2/16/99	Supervisor of the Ecogen-Monsanto research & development program
2/17/99 - present	Research Scientist, Monsanto

Research experience

My research has included the discovery of novel insecticidal protein genes from *Bacillus thuringiensis*, the engineering of improved insecticidal proteins, cloning vector design and construction, the development of an efficient gene transfer system for *B.thuringiensis* employing an indigenous site-specific recombination system, the study of DNA-protein interactions, the regulation of gene expression in Bacillus, and the optimization of *cry* gene expression in *B.thuringiensis*. This work led directly to the development of CRYMAX™ and Lepinox™, two new bioinsecticide products based on genetically-engineered *B.thuringiensis* strains and to patents covering improved strains of *B. thuringiensis*, new insecticidal proteins, and engineered insecticidal proteins. More recently, my research has included alternative insect control strategies employing RNA interference.

Education

1982-1987	Postgraduate	Postdoctoral research fellow with Drs. Norman H. Giles and Mary E. Case, University of Georgia, Athens, GA.

1981	Ph.D.	Genetics North Carolina State University, Raleigh, N.C. Doctoral Dissertation: "Genetic, Biochemical, and Developmental Studies of Maize Superoxide Dismutases."
1976	B.S.	Biology University of Notre Dame South Bend, IN. Honors: Magna cum laude, Phi Beta Kappa

Patents

Shuttle vector for recombinant *Bacillus thuringiensis* strain development. EP 0 533 701 B1. August, 24, 1994.

Bacillus thuringiensis transposon Tn*5401*. U. S. Patent No. 5,441,884. August 15, 1995.

Recombinant *Bacillus thuringiensis* strain construction method. U. S. Patent 5,650,308. July 22, 1997.

Recombinant *Bacillus thuringiensis* strains, insecticidal compositions, and methods of use. U. S. Patent 5,776,449. July 7, 1998.

Bacillus thuringiensis strains showing improved production of certain lepidopteran-toxic crystal proteins. U. S. Patent 5,804,180. September 8, 1998.

Bacillus thuringiensis Tn*5401* proteins. U. S. Patent 5,843,744. December 1, 1998.

Transgenic plants expressing lepidopteran-active δ-endotoxins. U. S. Patent 5,914,318. June 22, 1999.

Bacillus thuringiensis Cry1C compositions toxic to lepidopteran insects and methods for making Cry1C mutants. U. S. Patent 5,942, 664. August 24, 1999.

Chimeric lepidopteran-toxic crystal proteins. U. S. 5,965,428. October 12, 1999.

Polypeptide compositions toxic to *Diabrotica* insects, and methods of use [MECO 200]. WO 00/26378. Filed with U. S. PTO November 2, 1998.

Coleopteran-toxic polypeptide compositions and insect-resistant transgenic plants [MECO164]. WO 00/66742. Provisional filing with U. S. PTO May 4, 1999.

Lepidopteran-active *B. thuringiensis* δ-endotoxin compositions and methods of use [MECO201]. Provisional filing with U. S. PTO September 14, 1999.

Publications

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basis of the aggregation system in *B. thuringiensis* subsp. *israelensis* is located on the large conjugative plasmid pXO16. J. Bacteriol. 177:2914-2917.

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EXHIBIT B

CLAIMS PENDING AT TIME OF FEBRUARY 25, 2009, PATENT OFFICE COMMUNICATION

1-26. (Cancelled)

27. A method of making a nucleic acid comprising a coding sequence for expression in plant cells, said method comprising:

(a) starting with a coding sequence;

(b) modifying the coding sequence by substituting, for codons in the coding sequence, only codons for identical amino acids that have the highest frequency of use in plant genes, according to the plant codon usage table in Figure 1; and

(c) making a nucleic acid comprising the modified coding sequence that contains the substituted codons.

28. The method claim 27, wherein the modifying comprises substituting the codon with highest frequency of use for at least the first twenty-five amino acids of the starting coding sequence.

29. The method of claim 27, wherein the modifying comprises substituting the codon with highest frequency of use for at least 59 amino acids in the 5' end of the coding sequence.

30. The method of claim 27, further comprising attaching flanking regulatory sequences to the modified coding sequence.

31. The method of claim 27, wherein the starting coding sequence is a *Bacillus thuringiensis* (*B.t.*) coding sequence.

32. The method of claim 31, wherein the starting coding sequence codes for a *B.t.* delta endotoxin protein.

33. A method for constructing a nucleic acid comprising a protein coding sequence foreign to a plant cell and encoding a protein for expression in a plant cell, said method comprising

(a) starting with a protein coding sequence foreign to a plant cell;

(b) modifying the starting protein coding sequence of step (a) by substituting, for at least the first twenty-five amino acids of the starting protein coding sequence, a codon selected from Figure 1 that encodes the same amino acid and is used in the highest frequency in plants, and

(c) constructing a nucleic acid comprising a coding sequence containing the codons selected from Figure 1 and encoding the protein.

34. The method of claim 33, wherein the protein coding sequence encodes a prokaryotic or eukaryotic protein.

35. The method of claim 33, wherein the protein coding sequence encodes a *Bacillus thuringiensis* (*B.t.*) protein.

36. The method of claim 33, wherein the protein coding sequence encodes a *Bacillus thuringiensis* (*B.t.*) delta endotoxin.

37. The method of any one of claims 33-36, further comprising:

(d) attaching flanking regulatory sequences to the nucleic acid that comprises the coding sequence containing the codons selected from Figure 1 and encoding the protein.

38. A method for constructing a nucleic acid comprising a protein coding sequence foreign to a plant cell and encoding a protein for expression in a plant cell, said method comprising

(a) starting with a protein coding sequence foreign to a plant cell; and

(b) constructing a nucleic acid encoding the protein and containing codon substitutions relative to the starting protein coding sequence, wherein each codon substitution consists of a codon selected from Figure 1 that is used in the highest frequency in plants.

39. A method for constructing a nucleic acid comprising a protein coding sequence foreign to a plant cell and encoding a protein for expression in a plant cell, said method comprising

(a) starting with a protein coding sequence foreign to a plant cell; and

(b) constructing a nucleic acid encoding the protein and containing codon substitutions in the 5' end of the protein coding sequence relative to the starting protein coding sequence, wherein each of said codon substitutions in the 5' end consists of a codon selected from Figure 1 that is used in the highest frequency in plants.

40. A method for constructing a nucleic acid comprising a protein coding sequence foreign to a plant cell and encoding a protein for expression in a plant cell, said method comprising

(a) starting with a protein coding sequence foreign to a plant cell; and

(b) constructing a nucleic acid encoding the protein and containing codon substitutions in about the first 25 codons of the protein coding sequence relative to the starting protein coding sequence, wherein each of said codon substitutions in about the first 25 codons consists of a codon selected from Figure 1 that is used in the highest frequency in plants.

41. The method of any one of claims 38-40, wherein the protein coding sequence encodes a prokaryotic or eukaryotic protein.

42. The method of any one of claims 38-40, wherein the protein coding sequence encodes a *Bacillus thuringiensis* (*B.t.*) protein.

43. The method of any one of claims 38-40, wherein the protein coding sequence encodes a *Bacillus thuringiensis* (*B.t.*) delta endotoxin.

44. The method of any one of claims 38-40, further comprising:

(c) attaching flanking regulatory sequences to the nucleic acid that comprises the coding sequence containing the codons selected from Figure 1 and encoding the protein.